Spinal Fusion With Bone Morphogenetic Protein-2 Delivered by hydroxyapatite/collagen in a Rabbit

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Introduction: Posterolateral lumbar spinal fusion (PLF) is widely used for the treatment of degenerative lumbar diseases accompanied with instability. Although bone grafting of autologous iliac bone is commonly used for the PLF, the use of allografts or osteoinductive artificial grafts has increased because of morbidities associated with harvesting autologous bone. Demineralized bone matrix possesses some osteoinductivity, but its resulting bone fusion rates are inferior to those by autografts [1]. Therefore, artificial bone graft products supplemented with osteoinductive reagents such as bone morphogenetic proteins (eg, recombinant human bone morphogenetic protein-2 [rhBMP-2]) have been developed and applied clinically. However, there have not been BMP-carriers that fully satisfy the clinical demands, and therefore, high-dose BMP is needed to achieve satisfactory clinical results. As results, complications, which are thought to be caused by high-dose BMPs according to the insufficiency of the carriers, draws attentions.

We previously reported good osteochondral defect repair using fibroblast growth factor-2 (FGF-2) and a porous hydroxyapatite collagen composite (HAp/Col) that we originally developed as a bone void filler material [2]. HAp/Col consists of hydroxyapatite nanocrystals and type I atelocollagen, and it has high osteoconductivity, bio-absorbability, and sponge-like flexibility with superb handling properties. Furthermore, we have found that porous HAp/Col has a high affinity to BMPs, suggesting that porous HAp/Col is a suitable carrier for BMPs. These characteristics of HAp/Col may be effective for providing sustained release of BMPs.

Purpose of this study is to confirm the efficiency of the porous HAp/Col as a BMP-carrier using a rabbit PLF model.

Methods: The quantity of rhBMP-2 adsorbed on porous HAp/Col was determined. For adsorption, cylindrical porous HAp/Col (porosity: 95%, diameter: 5mm, height: 3mm) were impregnated with 40 µl of rhBMP-2 solution including 25 µg of rhBMP-2 and then incubated at 37°C for 30 minutes. The HAp/Col impregnated with BMP-2 was crushed in fetal bovine serum (FBS) by repeated compression, and the concentration of BMP-2 released from the HAp/Col into the serum was measured using ELISA. The total amount of BMP-2 that persisted in the HAp/Col after it was crushed was calculated.

All animal experiments were performed in accordance with the guidelines of Tokyo Medical and Dental University for the care and use of laboratory animals.
In this study, 32 skeletally mature male Japanese white rabbits (2.8-3.3 kg) were assigned into four group; a control group : porous HAp/Col (porosity: 95%, 10 x 30 x 5 mm) with 0, 36, 150 or 600 mg of rhBMP-2 dissolved in 0.5 mM HCl. Each solution was infiltrated into the implant 30 minutes prior to the transplantation.

Under general anesthesia, dorsal midline skin incision was made, and posterolateral spine fusion was performed between L4 and L5. After decortications of the transverse processes, the implants were transplanted bridging the transverse processes. The lumbar spine was harvested 6 weeks after the surgery and used for following evaluations. We evaluated by X-ray image, Micro-CT analysis, Manual palpation, Mechanical bending test, Histological evaluation.

Results: The locations of the transplanted sites were confirmed by bone formation between transverse processes in the X-ray images except for the control group (Fig. 1). No excessive bone formation which extended beyond the region of the transplanted implant was observed even in the 600 mg group. Fusion was confirmed by three-dimensional (3D) CT scanning. Bone formation and intertransverse fusion of each specimen was determined by axial images and sagittal images.

In control group, all cases didn’t show bone union. In 36 μg group, 6 cases showed bone union. In 150 μg and 600 μg group, all cases showed bone union. We could seem more new bone in 150μg and 600μg group than in 36μg group. To confirm the fixation, mobility between fixed vertebrae was tested by manual palpation test by two testers. The result of each case coincided between the testers without any exception. Except a case of the 36 mg group, all the cases of the BMP treated group were judged fused solid.

Using a mechanical testing machine, dorsal bending test was performed. At first, to stretch soft tissue, 100N was applied to the intervertebral disc. Then reset the position of the load cell head. Next, 300N (1mm/min) was applied. When facet was broken, we stopped the test. In control group , when flexed About 3mm , facet was broken(above figure). In the 36 μg group, the intervertebral disc was shifted 1.72 mm when 300N was applied. In the 150 μg and 600 mg group, the disc was shifted 1.73 and 1.53 mm respectively.(Fig.2)

We counted bone volume by micro CT,150 and 600 μg group were more new bone than 36 μg group. In histological study, all group showed new bone. But, control group showed no fusion.

The findings for 36μg, 150μg, 600μg group were similar. There was formation of new bone tissue and cartilaginous tissues. Around cartilaginous tissue , we could find osteoblast.

Discussion: Recombinant human bone morphogenetic protein-2 has been suggested as a replacement for autograft bone in spinal fusion treatments. However, its high cost and increased side effects have remained obstacles to its clinical application. Generally, clinical spinal fusion treatment requires several milligrams of rhBMP-2, which is a relatively high dosage for a growth factor. It was reported that the side effects of rhBMP-2 are closely related to its high dosage.

In this study, the PLF was performed without any additional internal fixation and demonstrated high fusion rate. PLF without internal fixation devices provided a more disadvantageous environment for fusion compared with clinical surgery, which uses internal fixation instruments such as pedicle screws or cages. Not only high performance of the porous HAp/Col as a BMP-carrier, from which the BMP release is thought to be sustained release, but also the sponge-like elasticity which resist pressure from surrounding muscle and flexibility to fit the curve of the transverse processes contributed the excellent result.
Significance: The study suggests that rhBMP-2 combined with porous Hap/col could be an alternative to autogenous bone grafting for posterolateral lumbar fusion of rabbits and potentially be applicable in a clinical setting.